

# Temperature sensitivity of ligand-gated ion channels: ryanodine receptor case

**B.I. Iaparov<sup>1,2</sup>, A.S. Moskvina<sup>1</sup>, O.E.Solovyova<sup>2,3</sup>**

<sup>1</sup> Department of Mathematical and Theoretical Physics, Institute of Natural Sciences and Mathematics, Ural Federal University, Ekaterinburg, Russia

<sup>2</sup> Laboratory of Mathematical Modelling in Physiology and Medicine, Ural Federal University, Ekaterinburg, Russia

<sup>3</sup> Laboratory of Mathematical Physiology, Institute of Immunology and Physiology, Ural Branch, Russian Academy of Sciences, Ekaterinburg, Russia

E-mail: bogdan.iaparov@urfu.ru

**Abstract.** Temperature influences all biochemical processes, in particular, excitation-contraction coupling (ECC) in cardiac cells. In this work we propose a theoretical explanation of temperature effects on an isolated ryanodine receptor calcium release channel (RyR channel) within the electron-conformational (EC) model. We show that the EC model with an Arrhenius-like temperature dependence of the "internal" and "external" frictions and a specific thermosensitivity of the tunnelling "open  $\leftrightarrow$  closed" transitions can provide both qualitative and quantitative description of the temperature effects for isolated RyR channels. Interestingly that a small change of the activation energy for the "internal" friction can make an ion channel either heat-inhibited or heat-activated while the "external" friction doesn't play a key role in temperature sensitivity: neglect of "external" friction doesn't change the channel's temperature sensitivity qualitatively.

## Introduction

Temperature influences all biochemical processes and the excitation-contraction coupling (ECC) in cardiac cells is not a special case. Experiments [1] show, that hypothermia increases action potential duration, intracellular  $\text{Ca}^{2+}$  transients and muscle contraction amplitude. The key elements of ECC are ryanodine receptors (RyRs), the ligand gated ion channels which govern the  $\text{Ca}^{2+}$  release from sarcoplasmic reticulum (SR), intracellular calcium storage. Abnormal RyR gating leads to cardiac arrhythmias and heart failure [2], hence the understanding the RyR gating is mandatory in order to understand the excitation-contraction coupling.

According to single RyR channel patch clamp experiments [3], the RyR channel gating changes significantly with temperature. Low temperatures ( $5^\circ\text{C}$ ) are characterized by longer openings ( $\langle t_{\text{open}} \rangle \approx 50 \text{ ms}$ ) with smaller ion current amplitude ( $\approx 2 \text{ pA}$ ) while high temperatures ( $23^\circ\text{C}$ ) are characterized by shorter openings ( $\langle t_{\text{open}} \rangle \approx 2 \text{ ms}$ ) with higher ion current amplitude ( $\approx 4 \text{ pA}$ ). From the viewpoint of the condensed matter physics it seems to be absolutely inexplicable how the  $\approx 6 \%$  change in absolute temperature can produce manifold effect in the channel activity. How can the temperature sensitivity of the RyR channel's gating be so high?



In this work we propose a theoretical explanation of the temperature effects for an isolated ryanodine receptor calcium release channel (RyR channel) within the electron-conformational(EC) model.

## Methods

### *Electron-conformational model*

Despite intensive structural studies of the RyR channels [4-8], our knowledge of molecular mechanisms for the RyR channel functioning is still limited. The RyR channel is the biggest known ion channel (more than 300000 atoms, molecular weight is  $\approx 2$  MDa) that makes very hard to study its molecular structure using both experimental methods such as low-temperature electron microscopy and theoretical methods such as molecular dynamics (MD).

Recently it was demonstrated that the electron-conformational model (see, e.g., Refs. [9, 10, 11]) is able to capture important features of the individual and cooperative behaviour of the RyRs in ventricular myocytes and pacemaker cells. The EC model of RyR functioning under  $\text{Ca}^{2+}$  stimuli is based on a biophysical adaptation of the well-known theory of photo-induced structural phase transitions, which has been successfully applied to different solids [12]. Within the EC model one start reducing a large variety of the RyR degrees of freedom to only two: a fast and a slow one, conditionally termed electronic and conformational, respectively. Bearing in mind the main function of the RyR channel as an ion channel, opening under stimuli and conducting ions, we assume only two actual electronic RyR channel states: open and closed, and a single conformational degree of freedom,  $Q$ , to be described by a classical continuous variable.

The key element of the EC model is the so called energetic approach, in other words, namely the energy is supposed to be a main characteristic of the RyR channels state. We introduce a simple effective Hamiltonian for an isolated RyR channel as follows:

$$H_s = -\Delta \hat{s}_z - h \hat{s}_x - pQ + \frac{K}{2} Q^2 + aQ \hat{s}_z, \quad (1)$$

where  $s_z$  and  $s_x$  are Pauli matrices, and the first term describes the bare energy splitting of "up" and "down" (electronically "open" and "closed") states with an energy gap  $\Delta$ , while the second term describes a quantum mixing effect. It is worth noting that given  $h = 0$  we arrive at a classical approach with a dichotomic electronic variable. The third and fourth terms in (1) describe the linear and quadratic contributions to the conformational energy. Here, the linear term formally corresponds to the energy of an external conformational stress, described by an effective stress parameter  $p$ . The last term describes the EC interaction with the coupling parameter  $a$ . Hereafter we make use of the dimensionless conformational variable  $Q$ ; therefore all of the model parameters ( $\Delta, h, p, K, a$ ) are assigned energy units. Two eigenvalues of our Hamiltonian

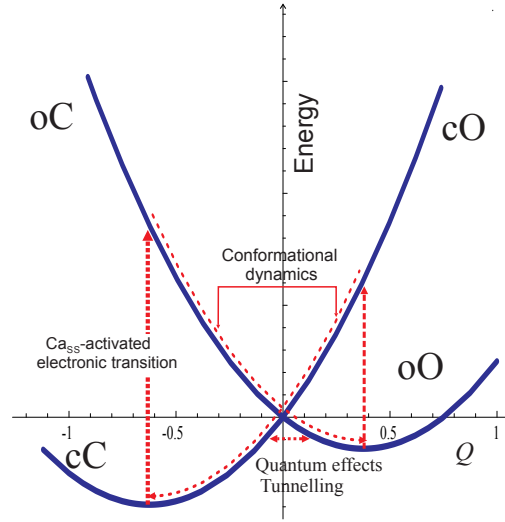
$$E_{\pm}(Q) = \frac{K}{2} Q^2 - pQ \pm \frac{1}{2} [(\Delta - aQ)^2 + h^2]^{1/2} \quad (2)$$

define two branches of the adiabatic, or conformational potential (CP), attributed to electronically closed ( $E_-$ ) and electronically open ( $E_+$ ) states of the RyR, respectively. Given  $h = 0$  we arrive at two diabatic potentials  $E_{\pm}(Q)$  for electronically closed and open states, shown in Fig. 1 at  $\Delta = 0$ . Two minima are separated by a intersection point at  $Q = 0$ .

### *Ion channel's dynamics in EC model*

The RyR channel's dynamics in EC model includes:

- Franck-Condon electronic transitions triggered by a subspace  $\text{Ca}^{2+}$  [9];



**Figure 1.** Conformational potential in the EC-model of the RyR channel given  $\hbar=0$  with a global minimum for a closed state. Small letters *c, o* are used for electronically closed and open states, respectively, and capital letters *C, O* for conformationally closed and fully open states, respectively. The energy separations  $\delta E_-$ ,  $\delta E_+$  are the potential barriers seen from the bottom of two diabatic potentials, respectively. Vertical arrows point to  $\text{Ca}^{2+}$ -induced Franck-Condon (FC) electronic transitions, horizontal arrow points to a non-FC tunneling transition, downhill arrows point to a conformational dynamics.

- Resonant quantum tunneling that takes place between two branches of the conformational potential with a probability obeyed the effective Gamov law as follows

$$P_{\text{tun}} = P_0 e^{-A_{\text{tun}} \Delta Q \sqrt{\Delta E}}, \quad (3)$$

where  $\Delta Q$  is the width,  $\Delta E$  is the height of the energy barrier, or the energy separation between the tunneling points and the intersection point (see Fig.1), and  $P_0$  effective tunneling attempt frequency;

- Slow conformational Langevin dynamics which does not obey the fluctuation-dissipation theorem and implies both internal or non-solvent friction (caused by interaction between RyR residues and interaction of RyR with the membrane) [13] and conventional thermal fluctuation forces:

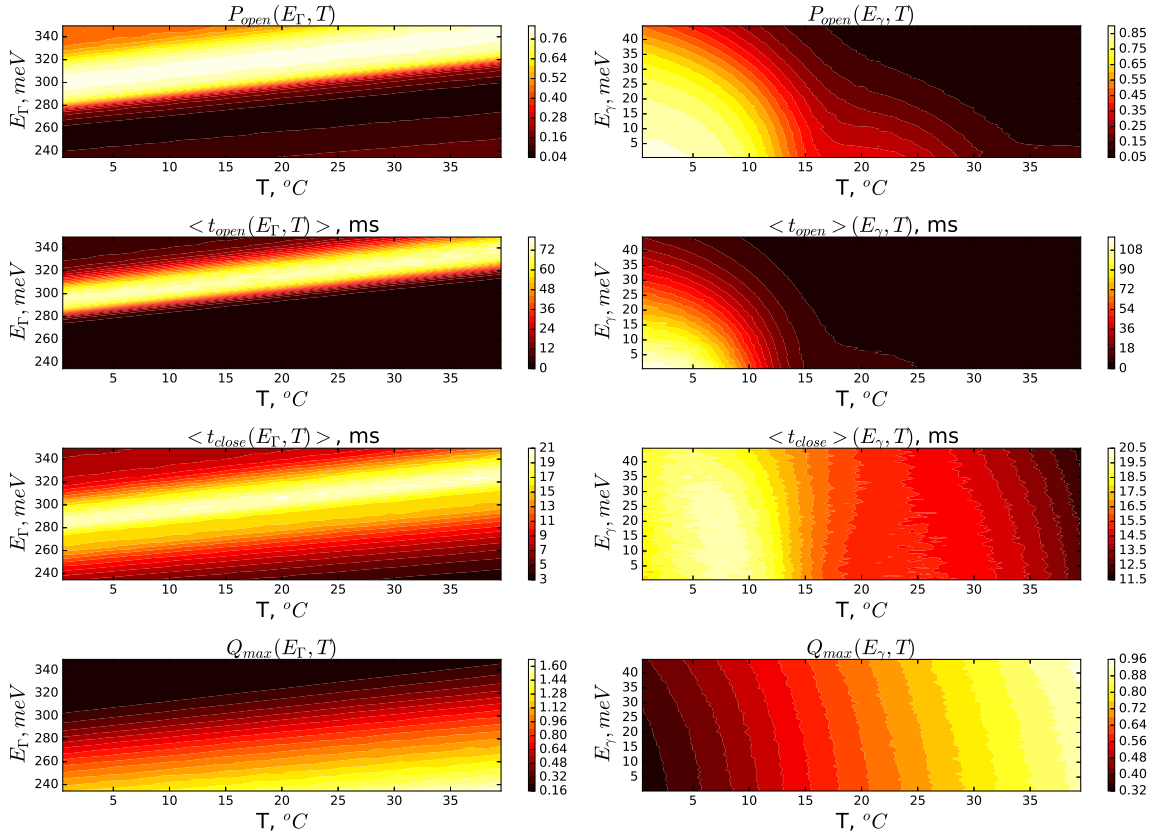
$$M\ddot{Q} = -\frac{\partial}{\partial Q}E(Q) - \Gamma\dot{Q} + \sqrt{2\gamma k_B T} R(t), \quad (4)$$

where first term describes a total systematic conformational force with  $M$  being an effective RyR mass (below  $M$  let to be unity),  $\Gamma$  is an effective dimensionless "internal" friction damping constant (relaxation rate). The last term describes the thermal fluctuation force (Gaussian-Markovian noise),  $\gamma$  is a random force parameter,  $T$  is a temperature,  $R(t)$  is a delta-correlated stationary Gaussian process with a zero-mean value.

Hereafter we neglect the temperature dependence of the main EC-model parameters except the friction parameter  $\Gamma$  and the random force parameter  $\alpha$ , these are assumed to obey the Arrhenius type temperature dependence:

$$\Gamma(T) = \Gamma_0 e^{\frac{E_\Gamma}{k_B T}}; \quad \gamma(T) = \gamma_0 e^{\frac{E_\gamma}{k_B T}} \quad (5)$$

with activation energies  $E_\Gamma$  and  $E_\gamma$ , respectively.



**Figure 2.** The temperature dependences for  $P_{open}$ ,  $\langle t_{open} \rangle$ ,  $\langle t_{close} \rangle$ , and maximal  $Q$  given different values of activation energies for internal  $E_{\Gamma}$  (left) and external  $E_{\gamma}$  (right) friction, respectively.

#### Conductance in EC model

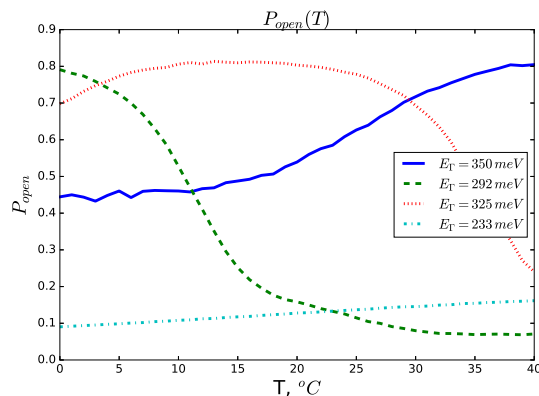
We assume the RyR channel's conductance to obey a simple power dependence

$$D(Q) = D Q^{\beta} \quad (6)$$

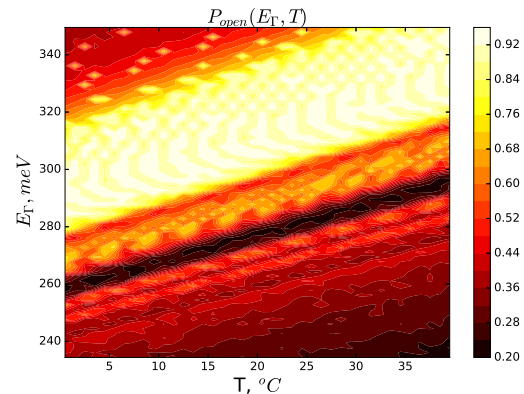
when RyR channel is electronically and conformationally open, otherwise  $D(Q) = 0$ .

#### Results

In our previous works [14, 15] we have shown that the features of the tunneling transitions between CP branches together with the concept of the effective temperature can provide a qualitative and quantitative explanation of the main temperature effects for the RyR gating. Hereafter, in the paper we focus on an influence of the activation energies  $E_{\Gamma}$  and  $E_{\gamma}$  on the temperature dependence of the main kinetic characteristics of the RyR channel gating, such as open time probability,  $P_{open}$ , mean open and mean closed times,  $\langle t_{open} \rangle$  and  $\langle t_{close} \rangle$ , respectively. We have performed a series of computer simulations of the RyR channel gating making use of a typical protocol of the EC model [9, 10, 11, 14] using the same set of parameters as in Ref. [15]. They were taken so that they provide a quantitative description of experimental data [3]. In Fig.2 we show the results of the  $(T, E_{\Gamma})$  and  $(T, E_{\gamma})$  parametric analysis for an isolated RyR channel. It is clearly seen that as a whole the change of  $E_{\Gamma}$  and  $E_{\gamma}$  shifts significantly the temperature dependence of the RyR channel kinetic characteristics ( $P_{open}$ ,  $\langle t_{open} \rangle$ ,  $\langle t_{close} \rangle$ ) and



**Figure 3.** Temperature dependence  $P_{open}$  if the external friction is neglected ( $\gamma_0 = 0$ ) given different  $E_\Gamma$ .



**Figure 4.** The temperature dependence  $P_{open}(E_\Gamma, T)$  if the external friction is neglected ( $\gamma_0 = 0$ ).

maximal  $Q$  ( $\langle Q_{max} \rangle$ ) which defines channel's conductance, though the effect of  $E_\gamma$  on  $\langle t_{close} \rangle$  and  $\langle Q_{max} \rangle$  seems to be marginal. It should be noted that the temperature dependence of main kinetic characteristics may be non-monotonic. Furthermore, the change of the activation energy  $E_\Gamma$ , in other words, the internal friction, can turn channel from the cold-activated to the heat-activated one with a significant change of the thermosensitivity  $Q_{10}$  (Fig.3). This theoretical result is partly supported by recent experimental data [16], which show that the changes in the membrane lipid composition, which certainly do influence the internal friction, markedly change the temperature behaviour of the RyR channels. Interestingly, the internal friction due to the RyR channel's inter-residue interactions seems to play a more important role in temperature effects as compared with the external friction. Indeed, Fig.4 shows that even if the external friction be neglected ( $\gamma_0 = 0$ ) the main kinetic characteristic  $P_{open}$  reveals a typical temperature behavior.

## Conclusion

We have demonstrated that a simple electron-conformational model with an assumption of an Arrhenius-type temperature dependences for the "internal" and "external" frictions, together with a specific "thermosensitivity" of the "open"  $\rightarrow$  "closed" tunneling probability [14, 15], is able to describe different types of the temperature behavior for the ion channel gating. Relatively small change of the activation energy  $E_\Gamma$  for the internal friction can turn channel from the cold-activated to the heat-activated one with a significant change of the thermosensitivity  $Q_{10}$ . We believe that similar mechanism can work for other ligand-gated ion channels.

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